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含 1,3,4-噻二唑和吡唑的酰胺衍生物的合成和抗肿瘤活性研究

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摘要 以苯乙酮和草酸二乙酯为原料, 设计并合成了一系列含有 1,3,4-噻二唑和吡唑的新型酰胺衍生物 **A1**～**A26**, 通过 ^{1}H NMR, ^{13}C NMR, IR, ESI-MS 和 HRMS 等多种手段对其结构进行了表征. 通过噻唑蓝(MTT)方法评估了这些化合物对人肝癌细胞(HepG2)和人胃癌细胞(MGC803)体外抗肿瘤活性的表现. 实验结果表明, 以 5-氟尿嘧啶作为阳性对照药物(IC_{50}=0.0787 μmol/mL), 化合物 **A1** (IC_{50}=0.0695 μmol/mL), **A2** (IC_{50}=0.0682 μmol/mL)和 **A3** (IC_{50}=0.0753 μmol/mL)对 HepG2 细胞表现出相似的抑制作用. 值得注意的是, 化合物 **A1** 对 MGC803 细胞表现出较强的抑制作用(IC_{50}=0.0420 μmol/ mL), 远比阳性对照药物 5-氟尿嘧啶(IC_{50}=0.0820 μmol/mL)优异.
关键词 酰胺衍生物; 有机合成; 1,3,4-噻二唑; 吡唑; 抗肿瘤活性

Synthesis and Antitumor Activity of Amide Derivatives Containing 1,3,4-Thiadiazole and Pyrazole Moieties

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Abstract In this work, a series of newly-synthesized amide derivatives (**A1**～**A26**) carrying 1,3,4-thiadiazole and pyrazole moieties were successfully designed and synthesized. In addition, their structures were characterized by multiple techniques including ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and HRMS. Furthermore, based on the antitumor results against human hepatocarcinoma cells (HepG2) and human gastric carcinoma cells (MGC803) via methyl thiazolyl tetrazolium (MTT) method, the *in vitro* cytotoxic activities of the compounds were evaluated, indicating that compounds **A1** (IC_{50}=0.0695 μmol/mL), **A2** (IC_{50}=0.0682 μmol/mL) and **A3** (IC_{50}=0.0753 μmol/mL) exhibited similar inhibitory effect against HepG2 cells. More importantly, compound **A1** displayed superior inhibition performance against MGC803 cells (IC_{50}=0.0420 μmol/mL) compared with that of 5-fluorouracil (IC_{50}=0.0820 μmol/mL).
Keywords amide derivatives; organic synthesis; 1,3,4-thiadiazole; pyrazole; antitumor activity

1 Introduction

Nitrogen-containing heterocycles compounds possessing 1,3,4-thiadiazole and pyrazole functional groups usually exhibit unique physiological and pharmacological properties.[1-6] For example, 1,3,4-thiadiazole compounds exhibit a broad range of novel biological activities such as anti-inflammatory,[7-8] antibacterial,[9-11] antiviral,[12] prevent depression,[13] anticonvulsant[14] and anticancer[15-19] activities. Pyrazole derivatives are of great importance in the field of pesticides and medicinal chemistry such as insecticidal activity,[19] anti-inflammatory,[20-21] anti-tuberculosis,[22-23] antifungal,[24-25] antiviral,[26-28] anticancer[29-33] activities. Owing to the advantages of low toxicity, high efficiency and the ability of multi-dimensional substitution by functional groups, 1,3,4-thiadiazole and pyrazole moieties-containing compounds play a vital role in the development of both pesticides and medicines.

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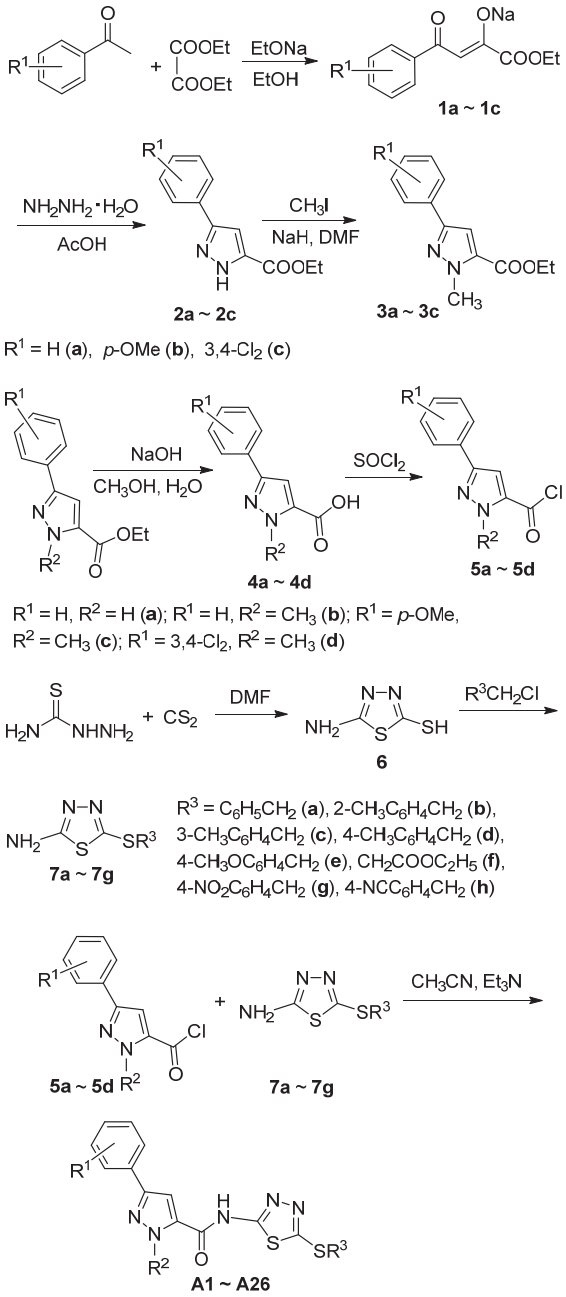
Through introduction of two heterocyclic active centers into the same molecule, the biological and physiological activities get further improved due to the synergistic interaction between different pharmacophores, which is conducive to the practical applications. Besides, the introduction of amide groups to drug molecules can facilitate the hydrogen bonds formation between biological macromolecules, leading to enhanced interaction force with receptors. Based on the principle of functions combination of diverse active groups in the drug molecule, we deliberately design the active amide structures containing 1,3,4-thiadiazole and pyrazole moieties together to further enhance their anticancer activities.

2 Results and discussion

2.1 Chemistry

The synthetic routes of the target compounds are depicted in Scheme 1. Substituted acetophenone and diethyl oxalate as starting materials underwent Claisen condensation of ester and Knorr cyclization to give ethyl 3-aryl-1*H*-5-pyrazolecarboxylate, and then underwent methylated reaction to generate the intermediates **3a**～**3c**. Hydrolysis reaction of compounds **3a**～**3c** led to the formation of 1-methyl-3-aryl-1*H*-5-pyrazolecarboxylic acids and 3-aryl-1*H*-5-pyrazolecarboxylic acids, which then reacted with thionyl chloride to yield pyrazole acid chloride intermediates **5a**～**5d**. The mixture of thiosemicarbazide and CS_2 was refluxed in *N*,*N*-dimethylformamide (DMF) to form 2-mercapto-5-amino-1,3,4-thiadiazole which then underwent nucleophilic substitution reaction with benzyl chloride to generate intermediates **7a**～**7g**. The nucleophilic substitution reaction between intermediates **5a**～**5d** and **7a**～**7g** yielded the target compounds **A1**～**A26**. All the newly-synthesized compounds were characterized using ^{1}H NMR and ^{13}C NMR, mass spectrometry and high resolution mass spectrometry (HRMS).

The intermediates 1,3-sodium diketone salts (**1a**～**1c**), which were converted to the corresponding 1,3-dicarbonyl compound under acidic conditions, were synthesized by substitution of acetophenone with diethyl oxalate as starting materials in a fresh ethanol solution of sodium ethoxide. In the Paal-Knorr reaction, a mixture of the sodium salts and hydrazine hydrate was refluxed in acetic acid to shorten the reaction cycle and improve the reaction yield via elimination of the conventional intermediates conversion step. When *p*-methoxyacetophenone or 3,4-dichloroacetophenone was used as starting materials, the NH containing pyrazole ring intermediates **2b**～**2c** were formed in the corresponding step. The resulting pyrazole acid chloride in the absence of *N*-methylation protection reacted with the substituted 2-amino-1,3,4-thiadiazole and no amide products were yielded. In repeated trials, the experiment was mediated by methyl iodide in dry DMF in the presence of NaH to produce *N*-methylated pyrazolecarboxylic acid, after which further acidification and reaction with thionyl chloride generate 1-methyl-3-aryl-1*H*-5-pyrazolecarboxylic acid chlorides **5b**～**5d**. Finally, the resulting acyl chlorides (**5a**～**5d**) reacted with a substituted 2-amino-1,3,4-thiadiazole derivatives (**7a**～**7g**) to afford the target compounds (**A1**～**A26**). Due to the formation of by-product HCl, triethylamine, a very necessary additive, was used as ac-



R^1 = H, R^2 = H: R^3 = $C_6H_5CH_2$ (**A1**), 2-$CH_3C_6H_4CH_2$ (**A2**), 3-$CH_3C_6H_4CH_2$ (**A3**), 4-$CH_3C_6H_4CH_2$ (**A4**), 4-$CH_3OC_6H_4CH_2$ (**A5**), $CH_2COOC_2H_5$ (**A6**), 4-$NO_2C_6H_4CH_2$ (**A7**), 4-$NCC_6H_4CH_2$ (**A8**)
R^1 = H, R^2 = CH_3: R^3 = $C_6H_5CH_2$ (**A9**), 2-$CH_3C_6H_4CH_2$ (**A10**), 4-$CH_3C_6H_4CH_2$ (**A11**), 4-$CH_3OC_6H_4CH_2$ (**A12**), 4-$NO_2C_6H_4CH_2$ (**A13**)
R^1 = *p*-OMe, R^2 = CH_3: R^3 = $C_6H_5CH_2$ (**A14**), 2-$CH_3C_6H_4CH_2$ (**A15**), 3-$CH_3C_6H_4CH_2$ (**A16**), 4-$CH_3C_6H_4CH_2$ (**A17**), 4-$CH_3OC_6H_4CH_2$ (**A18**), $CH_2COOC_2H_5$ (**A19**), 4-$NO_2C_6H_4CH_2$ (**A20**), 4-$NCC_6H_4CH_2$ (**A21**)
R^1 = 3,4-Cl_2, R^2 = CH_3: R^3 = $C_6H_5CH_2$ (**A22**), 2-$CH_3C_6H_4CH_2$ (**A23**), 3-$CH_3C_6H_4CH_2$ (**A24**), 4-$CH_3OC_6H_4CH_2$ (**A25**), 4-$NO_2C_6H_4CH_2$ (**A26**)

Scheme 1 Synthetic route of compounds **A1**～**A26**

id-binding agent. However, probably due to the p-π interaction between the lone pair of electrons in the amino nitrogen atom and π electrons of 1,3,4-thiadiazole ring, it made them difficult to react with acyl chlorides and the target products were obtained in moderate to good yields.

The infrared spectrum data of compounds **A1**～**A26** were recorded and analyzed by taking **A8** as an example. The broad peak at 3445 cm^{-1} is the stretching vibration absorption peak of N—H in the amide. The strong absorption at 3205 cm^{-1} is the N—H stretching vibration absorption peak on the pyrazole ring. The strong peak at 1663 cm^{-1} originates from the stretching vibration absorption peak of C=O. Due to the conjugation effect, the $\nu_{C=O}$ shifts to the lower wave number. The peak at 1625 cm^{-1} is attributed to stretching vibration absorption of C=N. The peaks at 1545 and 1456 cm^{-1} originate from the skeleton vibration absorption peaks of the aromatic ring.

The nuclear magnetic resonance spectrum data of compounds **A1**～**A26** were analyzed by taking **A12** ($C_{21}H_{19}N_5O_2S_2$) as an example. For ^1H NMR, the single peak at δ 13.20 is from the proton resonance peak of N—H in the amide. The 8 hydrogens of δ 7.79～7.35 in the range are from the resonance peaks of the protons on the aromatic ring. The peaks at δ 6.92～6.90 are attributed to the superpositions of the protons at the 4-position of the pyrazole ring and one of the protons at the aromatic ring. The single peak at δ 4.48 originates from the proton resonance peak of methylene group (SCH_2). The single peak at δ 4.18 is due to the resonance peak of CH_3 proton, and the single peak at δ 3.74 is the resonance peak of the OCH_3 proton on the benzene ring connected to the thiadiazole. For ^{13}C NMR, the peak at δ 159.6 corresponds to the carbon on the thiadiazole linked to the sulfur atom, and the peak at δ 159.3 is from the carbon on the carbonyl group attached to the pyrazole. The peak at δ 159.2 corresponds to the carbon on the benzene ring attached to the methoxy group, and the peak at δ 157.9 is from the carbon on the thiadiazole linked to N—H. The peaks at δ 134.8 and 149.1 are from the quaternary carbons on the pyrazole ring. The peaks at δ 132.6, 130.7, 129.4, 128.7, 128.5, 125.5 and 114.5 are the absorption peaks of carbons on the aromatic ring. The peak at δ 107.1 is the carbon at the 4-positions of pyrazole ring. The absorption peak at δ 37.7 is the carbon in the methyl group at the 1-position of pyrazole ring, δ 49.0 is the resonance peak of the methylene carbon attached to the sulfur atom, and δ 55.5 is the carbon of the methoxy group on the benzene ring.

The target compounds **A1**～**A26** were further confirmed by mass spectrometry and HRMS. In the ESI-MS spectrum, all target compounds gave $[M+H]^+$, and some compounds also showed $[M+Na]^+$, $[M+K]^+$ and $[M-H]^-$ peaks. In the HRMS spectrum, all target compounds gave $[M+H]^+$ peaks. The measured value is close to its calculated value and the error is negligible. Taking compound **A12** ($C_{21}H_{19}N_5O_2S_2$) as an example, the measured and calculated values of $[M+H]^+$ peak in HRMS are 438.1060 and 438.1058, respectively, with tiny difference of 0.0002. Within the allowable range, the structural correctness is proved.

2.2 Biological evaluation

In this paper, 5-fluorouracil was used as a positive control drug to study the growth inhibitory activity of target compounds on HepG2 and MGC-803 by methyl thiazolyl tetrazolium (MTT) assay. The growth inhibitory activity of the target product on HepG2 and MGC803 was determined by using 100 μg/mL as the primary screening concentration. The results are summarized in Table 1. The corresponding inhibition rate was calculated according to the related formula, as shown in Table 1. The activity test results showed that the target compounds had different degrees of inhibition on HepG2 at a concentration of 100 μg/mL. Among them, 11 compounds (**A1**, **A2**, **A3**, **A9**, **A11**, **A13**, **A14**, **A15**, **A16**, **A25** and **A26**) inhibited the proliferation of human hepatoma cells by more than 50%, and the anticancer activities range from moderate to good. The best activity, inhibition rate of 73.1%, was from compound **A2**. Compared to the antitumor activity of compound **A1**, it could be found that the compounds **A7** and **A8** reduced antitumor activities when R^1=H, R^2=H and R^3=4-NO_2-

Table 1 Inhibitory effects of compounds on human liver cancer cells (HepG2) and human gastric cancer cells (MGC803) at 100 μg/mL

Compd.	R^1	R^2	R^3	Inhibition rate/%		Compd.	R^1	R^2	R^3	Inhibition rate/%	
				HepG2	MGC803					HepG2	MGC803
A1	H	H	$C_6H_5CH_2$	50.6	54.2	**A14**	*p*-OMe	CH_3	$C_6H_5CH_2$	54.8	30.8
A2	H	H	2-$CH_3C_6H_4CH_2$	73.1	3.5	**A15**	*p*-OMe	CH_3	2-$CH_3C_6H_4CH_2$	53.2	31.8
A3	H	H	3-$CH_3C_6H_4CH_2$	66.2	15.3	**A16**	*p*-OMe	CH_3	3-$CH_3C_6H_4CH_2$	61.7	12.6
A4	H	H	4-$CH_3C_6H_4CH_2$	25.3	36.1	**A17**	*p*-OMe	CH_3	4-$CH_3C_6H_4CH_2$	2.8	47.4
A5	H	H	4-$CH_3OC_6H_4CH_2$	5.7	26.1	**A18**	*p*-OMe	CH_3	4-$CH_3OC_6H_4CH_2$	28.3	−9.0
A6	H	H	CH_2CO_2Et	35.8	42.6	**A19**	*p*-OMe	CH_3	CH_2CO_2Et	36.8	53.7
A7	H	H	4-$NO_2C_6H_4CH_2$	18.4	57.5	**A20**	*p*-OMe	CH_3	4-$NO_2C_6H_4CH_2$	37.6	49.3
A8	H	H	4-$NCC_6H_4CH_2$	16.1	45.9	**A21**	*p*-OMe	CH_3	4-$NCC_6H_4CH_2$	24.9	46.6
A9	H	CH_3	$C_6H_5CH_2$	54.4	26.0	**A22**	3,4-Cl_2	CH_3	$C_6H_5CH_2$	17.0	45.8
A10	H	CH_3	2-$CH_3C_6H_4CH_2$	42.9	50.3	**A23**	3,4-Cl_2	CH_3	2-$CH_3C_6H_4CH_2$	22.7	59.3
A11	H	CH_3	4-$CH_3C_6H_4CH_2$	59.8	44.8	**A24**	3,4-Cl_2	CH_3	3-$CH_3C_6H_4CH_2$	12.3	54.8
A12	H	CH_3	4-$CH_3OC_6H_4CH_2$	34.3	40.1	**A25**	3,4-Cl_2	CH_3	4-$CH_3OC_6H_4CH_2$	50.3	35.2
A13	H	CH_3	4-$NO_2C_6H_4CH_2$	60.6	27.7	**A26**	3,4-Cl_2	CH_3	4-$NO_2C_6H_4CH$	55.9	45.8

C_6H_4 or 4-NCC_6H_4. It was interesting that the introduction of a methyl group at the *ortho* and *meta* positions of the benzene ring as R^3 was important for improving antitumor activities and the antitumor activities of compounds **A1**～**A3** were found to be in the order of *ortho*->*meta*->*para*-. The similar trend can also be observed in **A14**～**A21**. When R^1=H, R^2=CH_3 and R^3=4-$NO_2C_6H_4$, it could be found that the antitumor activity of compound **A13** were better than those of compounds **A9**～**A12**. When R^1=3,4-Cl_2, R^2=CH_3 and R^3=4-$NO_2C_6H_4$, compound **A26** exhibited better antitumor activities than that of compounds **A22**～**A25**. From the general trend, it is favorable for the antitumor activity when both R^1 and R^2 were H.

The results also revealed that at a concentration of 100 μg/mL, compound **A18** showed a negative inhibitory effect and promoted the proliferation of MGC803 cells. Other target compounds exhibited different degrees of inhibition on MGC803, and the inhibition rates were between 12% and 60%. Among them, compounds **A1**, **A7**, **A10**, **A19**, **A23** and **A24** inhibited the proliferation of human gastric cancer cells by more than 50%, and the best inhibition rate 59.3% was from compound **A23**. From the inhibition rates of compounds **A1**～**A8**, compounds **A7** and **A8** showed obvious influence on the effect activity when R^1=H, R^2=H and R^3 was electron-withdrawing substituents 4-NO_2 or 4-CN on the benzene ring. When R^1=H and R^2=CH_3, compounds **A12** and **A13** with electron-donating methoxy group and electron-withdrawing nitro group of R^3 at the *para* position of the benzene ring showed reduced antitumor activities. When R^1=*p*-MeO, R^2=CH_3, and R^3 was ethyl acetate or an electron-withdrawing nitro group at the *para*-position of the benzene ring, compounds **A19** and **A20** showed good activity. Compounds **A15**～**A18** with electron-donating group methyl or methoxy showed negative inhibitory effect. From the overall trend, when R^1=3,4-Cl_2 and R^2=CH_3, compounds exhibited good antitumor activities against MGC803. It was of interest that the introduction of a methyl group to the benzene ring of R^3 in **A22**～**A26** was important for improving antitumor activities with the order of *ortho*->*meta*->*para*-.

For compounds exhibiting an inhibition rate greater than 50%, 5-fluorouracil was used as a positive control. 11 compounds were formulated into 6 different concentrations (64, 32, 16, 8, 4 and 2 μg/mL). According to the measured OD value, the half effective inhibitory concentration (IC_{50}) of each compound was calculated by SPSS 17.0 software. The results of IC_{50} values of samples to HepG2 and MGC-803 are summarized in Tables 2 and 3.

The results in Table 2 indicated that most of the compounds showed proliferation inhibition activity against HepG2, among which compounds **A1** (IC_{50}=0.0695 μmol/mL), **A2** (IC_{50}=0.0682 μmol/mL) and **A3** (IC_{50}=0.0753 μmol/mL) showed prominent anticancer activity against HepG2 cells, exceeding the positive control drug 5-fluorouracil (IC_{50}=0.0787 μmol/mL). Especially, compound **A2** (R^1=H, R^2=H and R^3=2-$CH_3C_6H_4CH_2$) was most excellent. Comparative analysis of compounds **A14**, **A15** and **A16** displayed that when R^1=*p*-OCH_3 and R^2=CH_3, compound **A16** (R^3=3-$CH_3C_6H_4CH_2$) has a certain inhibitory activity on HepG2. Nevertheless, compounds **A14** (R^3=$C_6H_5CH_2$) and **A15** (R^3=2-$CH_3C_6H_4CH_2$) showed no significant inhibitory effect on HepG2. From the above results, some structure-activity relationships could be concluded: (1) The compounds bearing *p*-OMe and 3,4-Cl_2 as R^1 showed negative inhibitory effect (Entries 7～11); (2) The introduction of a methyl group at R^2 reduced antitumor activities (Entry 4～11); (3) The methyl group as R^3 position on the benzene ring has no obvious influence on the effect activity, and similar results can also be observed (Entries 7～9).

Table 2 IC_{50} values of samples to human liver cancer cells (HepG2)[a]

Entry	Compd.	IC_{50}/(μmol•mL^{-1})
1	**A1**	0.0695
2	**A2**	0.0682
3	**A3**	0.0753
4	**A9**	3.0229
5	**A11**	—
6	**A13**	0.6020
7	**A14**	—
8	**A15**	—
9	**A16**	0.4666
10	**A25**	0.2553
11	**A26**	—
12	5-Fluorouracil	0.0787

[a] — indicates that there is basically no anti-cancer cell inhibitory activity.

Table 3 IC_{50} values of samples on human gastric cancer cells (MGC803)[a]

Entry	Compd.	IC_{50}/(μmol•mL^{-1})
1	**A1**	0.0420
2	**A7**	—
3	**A10**	—
4	**A19**	—
5	**A23**	—
6	**A24**	—
7	5-Fluorouracil	0.0820

[a] — indicates that there is basically no anti-cancer cell inhibitory activity.

The results in Table 3 demonstrated that only compound **A1** (R^1=H, R^2=H, R^3=$C_6H_5CH_2$) showed excellent inhibitory activity against MGC803 (IC_{50}=0.0420 μmol/mL) which obviously exceeded inhibitory activity of the positive control drug 5-fluorouracil (IC_{50}=0.0820 μmol/mL), while the other tested compounds showed no significant inhibitory effect on MGC803 (Entries 2～6). $C_6H_5CH_2$ as R^3 was crucial for inhibitory activities against MGC-803, while the other compounds bearing the other substituents as R^3 showed negative inhibitory effect. The introduction of *p*-OMe and 3,4-Cl_2 at R^1 and a methyl group at R^2 reduced antitumor activities.

3 Conclusions

In conclusion, we designed and synthesized a series of novel amide derivatives containing 1,3,4-thiadiazole and pyrazole derivatives. Besides, their structures were fully characterized and confirmed by IR, ^{1}H NMR, ^{13}C NMR, ESI-MS and HRMS *etc*. Evidenced by the *in vitro* antitumor activity of the target compounds against HepG2 and MGC803, some compounds exhibited a certain degree of inhibitory activities against HepG2 and MGC803. Among them, compounds **A1** (IC_{50}=0.0695 μmol/mL), **A2** (IC_{50}=0.0682 μmol/mL) and **A3** (IC_{50}=0.0753 μmol/mL) manifested similar inhibitory effect against HepG2 cells with 5-fluorouracil (IC_{50}=0.0787 μmol/mL). And compound **A1** showed the most potent inhibition against MGC803 cells (IC_{50}=0.0420 μmol/mL), significantly exceeding that of 5-fluorouracil (IC_{50}=0.0820 μmol/mL). Our research is expected to shed light on the study of structure-anticancer performance relationship. Other research on antibacterial and antiviral activities of these compounds is under the way.

4 Experimental section

4.1 Materials

All starting materials were commercially available and analytically pure. All manipulations were performed in air atmosphere. All chemicals and reagents were purchased from J&K Scientific Co., 9dingchem Scientific Co., and used without further purification. ^{1}H NMR spectra were recorded on a BRUKER DPX-400 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ and $(CD_3)_2SO$ as a solvent. Thin-layer chromatography (TLC) analysis was performed on silica gel Huanghai HSGF254 plates and visualized by quenching of UV fluorescence (λ_{max}=254 nm). Silica gel (200～300 mesh) was purchased from Qingdao Haiyang Chemical Co., China. Melting points were determined on an XT4A apparatus (Beijing Keyi Electro-optic Instrument Plant, China) and were uncorrected. The intensity data were collected on an Agilent xcalibur Eos-II-CCD-diffractometer (Agilent Technologies). FTIR spectra were obtained with a Bruker Tensor 27 instrument. All IR samples were prepared as thin films and reported in wave numbers (cm^{-1}). High-resolution mass spectra were performed on an ESI Q-TOF MS spectrometer (Micromass, England). Sodium benzoate ethyl pyruvate intermediates **1a**～**1c**,[34-36] 3-phenyl-1*H*-5-pyrazolecarboxylic acid ethyl ester intermediates **2a**～**2c**,[34-36] 1-methyl-3-phenyl-1*H*-5-pyrazolecarboxylic acid ethyl ester intermediates **3a**～**3c**,[34-36] 3-phenyl-1*H*-5-pyrazolecarboxylic acid intermediates **4a**～**4d**,[34-36] 3-phenyl-1*H*-5-pyrazolyl chloride intermediates **5a**～**5d**,[34-36] 2-mercapto-5-amino-1,3,4-thiadiazole **6**[37-38] and 2-benzylthio-5-amino-1,3,4-thiadiazole intermediates **7a**～**7g**[37-38] were synthesized according to the references.

4.2 General procedure

To a solution of 15 mL of anhydrous acetonitrile and 5 mL of triethylamine, 2-benzylthio-5-amino-1,3,4-thiadiazole (**7a**, 1.115 g, 5 mmol) was added in a 50 mL round bottom flask. The solution of the newly prepared acid chloride (**5a**, 10 mmol) in acetonitrile was added dropwise to the reaction mixture at 0 ℃. The mixture was heated to reflux for 5 h. The reaction was monitored by TLC.

The reaction mixture was then cooled to room temperature, diluted with water, extracted with ethyl acetate, washed with brine, cooled to 0 ℃, and filtered with suction. The crude product was washed with water and ethyl acetate to give compound **A1** as white solid (1.47 g, 75%). Compounds **A2**～**A26** were synthesized by this method.

4.3 Characterization data for products A1～A26

N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A1**): White powder, yield 75%. m.p. 266～267 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 14.15 (s, 1H, py-NH), 12.81 (s, 1H, CONH), 7.82 (d, J=6.6 Hz, 2H, ArH), 7.49 (d, J=8.4 Hz, 2H, ArH), 7.44～7.40 (m, 4H, ArH), 7.36～7.33 (m, 2H, ArH), 7.29 (s, 1H, py-CH), 4.52 (s 2H, SCH_2); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 162.2 160.9, 157.1, 141.9, 137.2, 135.7, 134.0, 129.9, 129.5, 129.0, 128.1, 125.8, 104.7, 38.1; IR (KBr) v: 3450, 1628, 1542, 1457, 1401, 1311, 1207, 1161, 1061, 758, 698 cm^{-1}; MS (ESI) m/z: 394.1 $[M+H]^+$, 392.0 $[M-H]^-$; HRMS calcd for $C_{19}H_{16}N_5OS_2$ 394.0796, found 394.0797.

N-(5-((2-Methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A2**): White powder, yield 69%. m.p. 270～271 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 14.17 (s, 1H, py-NH), 12.86 (s, 1H, CONH), 7.83 (d, J=7.2 Hz, 2H, ArH), 7.52～7.42 (m, 4H, ArH), 7.34 (d, J=7.3 Hz, 1H, ArH), 7.23～7.19 (m, 2H, ArH), 7.15 (s, 1H, py-CH), 4.54 (s, 2H, SCH_2), 2.40 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 159.7, 155.0, 152.8, 145.5, 137.3, 134.6, 130.9, 130.5, 129.6, 129.5, 129.0, 128.5, 127.7, 126.6, 125.8, 104.6, 36.6, 19.2; IR (KBr) v: 3450, 1674, 1627, 1543, 1456, 1401, 1303, 1161, 1057, 757, 670 cm^{-1}; MS (ESI) m/z: 408.0 $[M+H]^+$, 430.3 $[M+Na]^+$; HRMS calcd for $C_{20}H_{18}N_5OS_2$ 408.0953, found 408.0951.

N-(5-((3-Methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A3**): Light pink powder, yield 68%. m.p. 261 ～ 263 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 14.16 (s, 1H, py-NH), 12.83 (s, 1H, CONH), 7.83 (d, J=7.3 Hz, 2H, ArH), 7.51 (t, J=7.4 Hz, 2H, ArH), 7.42 (d, J=6.9 Hz, 2H, ArH), 7.25 (s, 1H, ArH), 7.23 (d, J=5.8 Hz, 2H, ArH), 7.10 (s, 1H, py-CH), 4.49 (s, 2H, SCH_2), 2.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 169.4, 160.6, 159.4, 145.5, 144.5, 138.2, 137.0, 130.1, 129.6, 129.3, 128.9, 128.7, 126.6, 125.9, 104.6, 38.0, 21.4; IR (KBr) v: 3451, 3299, 1685, 1626, 1544, 1457, 1401, 1316, 1208, 1161, 1060, 879, 760, 688 cm^{-1}; MS (ESI) m/z: 408.2 $[M+H]^+$, 430.2 $[M+Na]^+$; HRMS calcd for $C_{20}H_{18}N_5OS_2$ 408.0953, found 408.0952.

N-(5-((4-Methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A4**): White powder, yield 73%. m.p. 268～270 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 14.16 (s, 1H, py-NH), 12.82 (s, 1H, CONH), 7.82 (d, J=7.5 Hz, 2H, ArH), 7.49 (d, J=7.1 Hz, 2H, ArH), 7.34

(t, J=7.7 Hz, 4H, ArH), 7.16 (s, 1H, ArH), 7.14 (s, 1H, py-CH), 4.48 (s, 2H, SCH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.9, 164.0, 149.7, 149.3, 147.5, 146.0, 145.5, 140.2, 137.3, 134.0, 129.6, 129.4, 125.9, 101.5, 37.9, 21.2; IR (KBr) v: 3454, 1627, 1543, 1456, 1401, 1315, 1206, 1161, 1061, 911, 758, 674 cm^{-1}; MS (ESI) m/z: 408.2 $[M+H]^+$, 430.1 $[M+Na]^+$; HRMS calcd for $C_{20}H_{18}N_5OS_2$ 408.0953, found 408.0955.

N-(5-((4-Methoxybenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A5**): White powder, yield 62%. m.p. 269～271 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.16 (s, 1H, py-NH), 12.82 (s, 1H, CONH), 7.83 (d, J=7.5 Hz, 2H, ArH), 7.53～7.41 (m, 4H, ArH), 7.36 (d, J=8.5 Hz, 2H, ArH), 6.92 (s, 1H, ArH), 6.89 (s, 1H, py-CH), 4.47 (s, 2H, SCH_2), 3.74 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 165.5, 163.0, 159.2, 153.3, 147.4, 144.5, 130.8, 129.7, 129.3, 128.8, 125.9, 114.4, 104.6, 55.5, 37.6; IR (KBr) v: 3449, 3301, 2833, 1686, 1626, 1545, 1513, 1463, 1400, 1316, 1207, 1174, 1062, 879, 761, 637 cm^{-1}; MS (ESI) m/z: 424.3 $[M+H]^+$, 446.2 $[M+Na]^+$; HRMS calcd for $C_{20}H_{18}N_5O_2S_2$ 424.0902, found 424.0899.

Ethyl 2-((5-(3-phenyl-1*H*-pyrazole-5-carboxamido)-1,3,4-thiadiazol-2-yl)thio)acetate (**A6**): White powder, yield 57%. m.p. 265～267 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.16 (s, 1H, py-NH), 12.85 (s, 1H, CONH), 7.83 (d, J=7.2 Hz, 2H, ArH), 7.51 (t, J=7.3 Hz, 2H, ArH), 7.42 (d, J=3.9 Hz 2H, ArH+py-CH), 4.20 (s, 2H, SCH_2), 4.15 (q, J=7.1 Hz, 2H, OCH_2CH_3), 1.20 (t, J=7.1 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.7, 160.5, 145.8, 136.4, 134.8, 130.5, 130.2, 129.6, 127.7, 125.8, 104.7, 61.8, 35.6, 14.4; IR (KBr) v: 3449, 3297, 3130, 1728, 1682, 1629, 1542, 1400, 1300, 1208, 1175, 1065, 760, 692 cm^{-1}; MS (ESI) m/z: 390.0 $[M+H]^+$, 412.2 $[M+Na]^+$, 428.2 $[M+K]^+$; HRMS calcd for $C_{16}H_{16}N_5O_3S_2$ 390.0695, found 390.0693.

N-(5-((4-Nitrobenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A7**): White powder, yield 55%. m.p. 273～275 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.15 (s, 1H, py-NH), 12.84 (s, 1H, CONH), 7.83～7.80 (m, 4H, ArH), 7.62 (d, J=7.9 Hz, 2H, ArH), 7.48～7.40 (m, 4H, ArH+py-CH), 4.59 (s, 2H, SCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.8, 158.0, 154.7, 147.2, 145.8, 145.5, 144.5, 130.8, 129.6, 128.7, 125.9, 124.1, 123.5, 104.8, 37.0; IR (KBr) v: 3432, 3195, 2852, 1674, 1618, 1554, 1520, 1456, 1401, 1345, 1293, 1052, 878, 760, 691 cm^{-1}; IR (KBr) v: 3438, 3334, 2930, 2856, 1616, 1452, 1402, 1225, 1006, 846, 782 cm^{-1}; MS (ESI) m/z: 437.2 $[M-H]^-$; HRMS calcd for $C_{19}H_{15}N_6O_3S_2$ 439.0647, found 439.0644.

N-(5-((4-Cyanobenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A8**): White powder, yield 63%. m.p. 269～271 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.16 (s, 1H, py-NH), 12.86 (s, 1H, CONH), 8.21 (d, J=8.6 Hz, 2H, ArH), 7.83 (d, J=7.8 Hz, 2H, ArH), 7.71 (d, J=8.6 Hz, 2H, ArH), 7.53～7.47 (m, 2H, ArH), 7.41 (d, J=3.8 Hz 2H, ArH+py-CH), 4.67 (s, 2H, SCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.4, 160.6, 157.3, 155.9, 155.4, 152.1, 132.9, 130.5, 129.6, 128.8, 125.7, 124.9, 115.2, 114.4, 104.5, 37.3; IR (KBr) v: 3445, 3205, 1663, 1625, 1545, 1456, 1401, 1312, 1162, 634 cm^{-1}; MS (ESI) m/z: 419.5 $[M+H]^+$, 441.3 $[M+Na]^+$; HRMS calcd for $C_{20}H_{15}N_6OS_2$ 419.0749, found 419.0748.

N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-1-methyl-3-phenyl-1*H*-pyrazole-5-carboxamide (**A9**): Light yellow powder, yield 62%. m.p. 243～246 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.21 (s, 1H, NH), 7.98 (d, J=7.1 Hz, 3H, ArH), 7.49～7.43 (m, 4H, ArH), 7.39～7.34 (m, 3H, ArH), 7.30 (d, J=7.1 Hz, 1H, py-CH), 4.54 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.6, 159.2, 157.9, 149.1, 137.0, 134.7, 132.6, 129.5, 129.4, 129.0, 128.5, 128.1, 125.5, 107.1, 45.0, 38.0; IR (KBr) v: 3448, 1666, 1626, 1530, 1455, 1401, 1289, 694 cm^{-1}; MS (ESI) m/z: 408.4 $[M+H]^+$, 430.2 $[M+Na]^+$, 446.3 $[M+K]^+$; HRMS calcd for $C_{20}H_{18}N_5OS_2$ 408.0953, found 408.0951.

1-Methyl-*N*-(5-((2-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A10**): Off-white powder, yield 65%. m.p. 244～246 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.22 (s, 1H, NH), 7.79 (d, J=7.2 Hz, 3H, ArH), 7.47 (t, J=7.6 Hz, 2H, ArH), 7.39～7.34 (m, 2H, ArH), 7.23 (t, J=7.6 Hz, 2H, ArH), 7.18～7.14 (m, 2H, ArH+py-CH), 4.55 (s, 2H,SCH_2), 4.18 (s, 3H, py-CH_3), 2.40 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.7, 159.0, 156,2, 149.1, 137.3, 134.8, 134.5, 132.6, 130.9, 130.4, 129.4, 128.5, 126.6, 125.5, 123.4, 107.1, 49.1, 36.7, 19.2; IR (KBr) v: 3448, 3207, 3124, 1667, 1520, 1456, 1402, 1259, 1060, 883, 734, 688 cm^{-1}; MS (ESI) m/z: 422.5 $[M+H]^+$; HRMS calcd for $C_{21}H_{20}N_5OS_2$ 422.1109, found 422.1108.

1-Methyl-*N*-(5-((4-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A11**): Brown powder, yield 73%. m.p. 245 ～ 246 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.17 (s, 1H, NH), 7.78 (d, J=7.8 Hz, 3H, ArH), 7.47 (t, J=7.6 Hz, 2H, ArH), 7.38 (d, J=7.3 Hz, 1H, ArH), 7.32 (d, J=7.9 Hz, 2H, ArH), 7.16 (d, J=7.8 Hz, 2H, ArH+py-CH), 4.49 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3), 2.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 165.9, 159.6, 159.3, 149.1, 137.4, 134.8, 134.5, 133.9, 132.6, 129.6, 129.4, 128.5, 125.5, 107.1, 49.1, 37.8, 21.2; IR (KBr) v: 3449, 1668, 1627, 1520, 1455, 1401, 1293, 1247, 884, 767, 691 cm^{-1}; MS (ESI) m/z: 422.1 $[M+H]^+$, 444.3 $[M+Na]^+$, 460.3 $[M+K]^+$; HRMS calcd for $C_{21}H_{20}N_5OS_2$ 422.1109, found 422.1111.

1-Methyl-*N*-(5-((4-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A12**): Light yellow powder, yield 78%. m.p. 243～245 ℃; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.20 (s, 1H, NH), 7.78 (d, J=7.6 Hz, 3H, ArH), 7.47 (t, J=7.5 Hz, 2H, ArH), 7.36 (d, J=8.4 Hz, 3H, ArH), 6.91 (d, J=8.6 Hz, 2H, ArH+py-CH), 4.48 (s, 2H, SCH_2), 4.18 (s, 3H, py-CH_3), 3.74 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.6, 159.3, 159.2, 157.9, 149.1, 134.8, 132.6, 130.7, 129.4, 128.7, 128.5, 125.5, 114.5, 107.1, 55.5, 49.0, 37.7; IR (KBr) v: 3443, 3204, 1663, 1618, 1517, 1455, 1401, 1297, 1248, 1173, 1034, 738, 691 cm^{-1}; MS (ESI) m/z: 438.3 $[M+H]^+$, 460.2 $[M+Na]^+$, 476.2 $[M+K]^+$; HRMS calcd for $C_{21}H_{20}N_5O_2S_2$ 438.1058,

found 438.1060.

1-Methyl-*N*-(5-((4-nitrobenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-phenyl-1*H*-pyrazole-5-carboxamide (**A13**): Light yellow powder, yield 70%. m.p. 251～253 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.20 (s, 1H, NH), 8.21 (d, J=8.6 Hz, 2H, ArH), 7.78 (d, J=7.9 Hz, 3H, ArH), 7.38 (d, J=8.6 Hz, 2H, ArH), 7.47 (t, J=7.6 Hz, 2H, ArH), 7.36 (t, J=7.2 Hz, 1H, py-CH), 4.68 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 162.1, 158.8, 158.3, 149.0, 147.3, 145.7, 132.6, 130.7, 129.4, 125.4, 124.1, 112.0, 108.2, 107.1, 49.1, 37.0; IR (KBr) v: 3447, 3111, 2947, 1668, 1610, 1521, 1455, 1403, 1298, 1098, 776, 694 cm^{-1}; MS (ESI) m/z: 453.2 $[M+H]^+$, 451.2 $[M-H]^-$; HRMS calcd for $C_{20}H_{17}Cl_2N_6O_3S_2$ 453.0804, found 453.0805.

N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-3-(4-methoxyphenyl)-1-methyl-1*H*-pyrazole-5-carboxamide (**A14**): Light yellow powder, yield 73%. m.p. 243～245 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.14 (s, 1H, NH), 7.69 (t, J=7.9 Hz, 3H, ArH), 7.44 (d, J=7.4 Hz, 2H, ArH), 7.35 (t, J=7.4 Hz, 2H, ArH), 7.30 (d, J=7.2 Hz, 1H, ArH), 7.03 (d, J=8.7 Hz, 2H, ArH+py-CH), 4.54 (s, 2H, SCH_2), 4.15 (s, 3H, py-CH_3), 3.80 (s, 2H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 160.9, 159.7, 159.1, 149.0, 147.9, 137.1, 130.8, 129.4, 129.0, 128.1, 126.8, 125.2, 114.8, 106.5, 55.6, 49.1, 38.0; IR (KBr) v: 3446, 3207, 1664, 1622, 1528, 1454, 1402, 1293, 1249, 1171, 1036, 705 cm^{-1}; MS (ESI) m/z: 438.2 $[M+H]^+$, 460.1 $[M+Na]^+$; HRMS calcd for $C_{21}H_{20}N_5O_2S_2$ 438.1058, found 438.1056.

3-(4-Methoxyphenyl)-1-methyl-*N*-(5-((2-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide (**A15**): White powder, yield 63%. m.p. 244～245 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.17 (s, 1H, NH), 7.70 (t, J=8.8 Hz, 3H, ArH), 7.22 (d, J=7.3 Hz, 1H, ArH), 7.22～7.21 (m, 2H, ArH), 7.17～7.13 (m, 1H, ArH), 7.03 (d, J=8.8 Hz, 2H, ArH+py-CH), 4.54 (s, 2H, SCH_2), 4.14 (s, 3H, py-CH_3), 3.79 (s, 2H, OCH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.7, 158.9, 149.0, 137.3, 134.5, 130.9, 130.4, 128.5, 126.8, 126.6, 125.2, 122.5, 114.8, 106.5, 55.6, 39.1, 36.7, 19.2; IR (KBr) v: 3440, 3206, 1664, 1620, 1522, 1454, 1402, 1294, 1249, 1171, 1038, 731 cm^{-1}; MS (ESI) m/z: 452.3 $[M+H]^+$, 474.2 $[M+Na]^+$; HRMS calcd for $C_{22}H_{22}N_5O_2S_2$ 452.1215, found 452.1211.

3-(4-Methoxyphenyl)-1-methyl-*N*-(5-((3-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide **A16**: Light gray powder, yield 69%. m.p. 226～228 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.15 (s, 1H, NH), 7.69 (d, J=8.8 Hz, 3H, ArH), 7.23 (t, J=6.4 Hz, 3H, ArH), 7.10 (d, J=5.9 Hz, 1H, ArH) , 7.02 (d, J=8.8 Hz, 2H, ArH+py-CH), 4.49 (s, 2H, SCH_2), 4.14 (s, 3H, py-CH_3), 3.79 (s, 2H, OCH_3), 2.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 159.6, 159.3, 157.9, 155.2, 149.0, 138.2, 136.8, 134.6, 130.1, 128.9, 128.8, 126.8, 126.6, 125.2, 114.8, 106.5, 55.6, 38.4, 38.0, 21.4; IR (KBr) v: 3445, 1663, 1622, 1528, 1455, 1402, 1290, 1249, 1170, 1037, 729 cm^{-1}; MS (ESI) m/z: 452.3 $[M+H]^+$, 474.2 $[M+Na]^+$, 490.1 $[M+K]^+$; HRMS calcd for $C_{22}H_{22}N_5O_2S_2$ 452.1215, found 452.1213.

3-(4-Methoxyphenyl)-1-methyl-*N*-(5-((4-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide (**A17**): Light brown powder, yield 71%. m.p. 245～247 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.18 (s, 1H, NH), 7.69 (t, J=6.2 Hz, 3H, ArH), 7.32 (d, J=7.9 Hz, 2H, ArH), 7.16 (d, J=7.9 Hz, 2H, ArH), 7.11 (d, J=8.8 Hz, 2H, ArH+py-CH), 4.49 (s, 2H, SCH_2), 4.15 (s, 3H, py-CH_3), 3.80 (s, 2H, OCH_3), 2.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.0, 164.6, 159.6, 158.9, 145.2, 141.5, 139.3, 136.1, 129.6, 129.4, 128.0, 126.8, 122.0, 114.8, 55.6, 49.1, 37.8, 21.2; IR (KBr) v: 3448, 1670, 1624, 1548, 1520, 1449, 1400, 1290, 1251, 1174, 1037, 835, 732 cm^{-1}; MS (ESI) m/z: 452.3 $[M+H]^+$, 474.2 $[M+Na]^+$; HRMS calcd for $C_{22}H_{22}N_5O_2S_2$ 452.1215, found 452.1212.

N-(5-((4-Methoxybenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-(4-methoxyphenyl)-1-methyl-1*H*-pyrazole-5-carboxamide (**A18**): Light gray powder, yield 60%. m.p. 248～249 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.16 (s, 1H, NH), 7.67 (t, J=6.0 Hz, 3H, ArH), 7.35 (d, J=8.6 Hz, 2H, ArH), 7.02 (d, J=8.8 Hz, 2H, ArH) , 6.90 (d, J=8.6 Hz, 2H, ArH+py-CH), 4.48 (s, 2H, SCH_2), 4.14 (s, 3H, py-CH_3), 3.79 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 161.5, 159.6, 159.2, 158.0, 149.0, 138.6, 134.7, 130.7, 128.7, 126.8, 125.3, 114.8, 114.5, 106.5, 55.6, 55.6, 39.0, 37.7; IR (KBr) v: 3450, 1663, 1620, 1516, 1454, 1402, 1295, 1247, 1175, 1034, 833, 732 cm^{-1}; MS (ESI) m/z: 466.2 $[M-H]^-$; HRMS calcd for $C_{22}H_{22}N_5O_3S_2$ 468.1164, found 468.1165.

Ethyl 2-((5-(3-(4-methoxyphenyl)-1-methyl-1*H*-pyrazole-5-carboxamido)-1,3,4-thiadiazol-2-yl)thio)acetate (**A19**): Brown powder, yield 68%. m.p. 201～202 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.17 (s, 1H, NH), 7.69 (d, J=8.6 Hz, 3H, ArH), 7.02 (d, J=8.8 Hz, 2H, ArH+py-CH), 4.21 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3), 4.14 (q, J=7.0 Hz, 2H, OCH_2CH_3), 3.79 (s, 3H, OCH_3), 1.19 (t, J=7.1 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.5, 168.6, 161.8, 159.8, 159.6, 149.0, 141.1, 139.7, 134.5, 126.8, 114.7, 61.8, 55.6, 35.6, 30.6, 14.4; IR (KBr) v: 3432, 3164, 2841, 1721, 1668, 1614, 1547, 1517, 1452, 1402, 1293, 1248, 1178, 1028, 882, 830 cm^{-1}; MS (ESI) m/z: 434.3 $[M+H]^+$, 456.2 $[M+Na]^+$, 472.1 $[M+K]^+$; HRMS calcd for $C_{18}H_{20}N_5O_4S_2$ 434.0957, found 434.0951.

3-(4-Methoxyphenyl)-1-methyl-*N*-(5-((4-nitrobenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide (**A20**): Brown powder, yield 55%. m.p. 257～259 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.20 (s, 1H, NH), 8.21 (d, J=8.8 Hz, 2H, ArH), 7.72～7.67 (m, 5H, ArH), 7.03 (d, J=8.8 Hz, 2H, ArH+py-CH), 4.68 (s, 2H, SCH_2), 4.14 (s, 3H, py-CH_3), 3.80 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 160.1, 159.6, 158.2, 158.0, 149.0, 147.2, 145.6, 134.7, 130.7, 126.8, 125.2, 124.1, 114.8, 106.5, 55.6, 37.0, 34.0; IR (KBr) v: 3447, 3201, 1668, 1618, 1523, 1455, 1402, 1297, 1249, 1174, 1032, 730 cm^{-1}; MS (ESI) m/z: 483.2 $[M+H]^+$, 505.0 $[M+Na]^+$; HRMS calcd for $C_{21}H_{19}N_6O_4S_2$ 483.0909, found 483.0909.

N-(5-((4-Cyanobenzyl)thio)-1,3,4-thiadiazol-2-yl)-3-(4-methoxyphenyl)-1-methyl-1*H*-pyrazole-5-carboxamide (**A21**): Brown powder, yield 58%. m.p. 263～264 ℃; ^{1}H

NMR (DMSO-d_6, 400 MHz) δ: 13.18 (s, 1H, NH), 7.82 (d, J=8.2 Hz, 2H, ArH), 7.68 (t, J=6.3 Hz, 3H, ArH), 7.63 (d, J=8.2 Hz, 2H, ArH), 7.02 (d, J=8.8 Hz, 2H, ArH+py-CH), 4.61 (s, 2H, SCH_2), 4.14 (s, 3H, py-CH_3), 3.79 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.6, 164.4, 154.0, 147.1, 145.1, 138.8, 136.0, 134.9, 132.9, 130.4, 126.8, 121.3, 114.8, 110.8, 101.0, 60.8, 55.5, 48.6; IR (KBr) v: 3446, 3206, 1669, 1622, 1515, 1450, 1400,1304, 1291, 1249, 1172, 601 cm^{-1}; MS (ESI) m/z: 463.3 $[M+H]^+$, 485.0 $[M+Na]^+$; HRMS calcd for $C_{21}H_{19}N_6O_3S_2$ 463.1011, found 463.1008.

N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-3-(3,4-dichlorophenyl)-1-methyl-1*H*-pyrazole-5-carboxamide (**A22**): Off-white powder, yield 56%. m.p. 258～259 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.22 (s, 1H, NH), 7.96 (s, 1H, ArH), 7.82 (s, 1H, ArH), 7.74 (s, 2H, ArH), 7.44 (d, J=7.2 Hz, 2H, ArH), 7.35 (t, J=7.3 Hz, 2H, ArH), 7.30 (d, J=7.2 Hz, 1H, py-CH), 4.54 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 164.5, 156.3, 146.6, 144.1, 137.0, 135.4, 133.2, 132.2, 131.7, 130.9, 129.4, 129.1, 128.1, 127.0, 125.5, 107.7, 38.0, 37.7; IR (KBr) v: 3444, 3203, 1667, 1624, 1543, 1530, 1455, 1435, 1401, 1291, 885, 728, 706 cm^{-1}; MS (ESI) m/z: 476.2 $[M+H]^+$. HRMS calcd for $C_{20}H_{16}Cl_2N_5OS_2$ 476.0173, found 476.0172.

3-(3,4-Dichlorophenyl)-1-methyl-*N*-(5-((2-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide (**A23**): Brown powder, yield 59%. m.p. 256～258 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.24 (s, 1H, NH), 7.95 (s, 1H, ArH), 7.81 (s, 1H, ArH), 7.73 (s, 2H, ArH), 7.34 (d, J=7.4 Hz, 1H, ArH), 7.22 (t, J=2.4 Hz, 2H, ArH), 7.17～7.13 (m, 1H, py-CH), 4.54 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.1, 159.3, 148.8, 147.4, 139.3, 138.4, 137.3, 134.6, 132.2, 131.8, 130.9, 130.4, 128.5, 127.0, 126.6, 125.5, 118.7, 107.7, 36.6, 35.5, 19.2; IR (KBr) v: 3448, 3208, 1669, 1626, 1518, 1459, 1400, 1293, 1261, 1159, 727 cm^{-1}; MS (ESI) m/z: 490.1 $[M+H]^+$, 512.1 $[M+Na]^+$, 528.0 $[M+K]^+$; HRMS calcd for $C_{21}H_{18}Cl_2N_5OS_2$ 490.0330, found 490.0327.

3-(3,4-Dichlorophenyl)-1-methyl-*N*-(5-((3-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide (**A24**): White powder, yield 55%. m.p. 254～256 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.23 (s, 1H, NH), 7.96 (s, 1H, ArH), 7.82 (s, 1H, ArH), 7.74 (s, 2H, ArH), 7.24 (t, J=6.5 Hz, 3H, ArH), 7.11 (d, J=6.0 Hz, 1H, py-CH), 4.50 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 162.8, 160.9, 146.7, 144.0, 138.2, 136.8, 133.5, 133.2, 132.2, 131.8, 130.9, 130.1, 129.0, 128.8, 127.0, 126.5, 125.5, 107.7, 41.3, 38.0, 21.4; IR (KBr) v: 3450, 3212, 1668, 1626, 1532, 1461, 1436, 1402, 1292, 885, 729 cm^{-1}; MS (ESI) m/z: 490.2 $[M+H]^+$, 512.3 $[M+Na]^+$, 528.1 $[M+K]^+$; HRMS calcd for $C_{21}H_{18}Cl_2N_5OS_2$ 490.0330, found 490.0329.

3-(3,4-Dichlorophenyl)-*N*-(5-((4-methoxybenzyl)thio)-1,3,4-thiadiazol-2-yl)-1-methyl-1*H*-pyrazole-5-carboxamide (**A25**): Off-white powder, yield 49%. m.p. 248～250 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.23 (s, 1H, NH), 7.96 (s, 1H, ArH), 7.82 (s, 1H, ArH), 7.74 (s, 2H, ArH), 7.36 (d, J=8.6 Hz, 2H, ArH), 6.91 (d, J=8.6 Hz, 2H, ArH+py-CH), 4.48 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3), 3.74 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 164.9, 159.4, 159.2, 152.3, 146.7, 133.2, 132.2, 131.9, 131.7, 130.7, 128.7, 127.0, 125.5, 114.4, 107.7, 55.5, 49.1, 37.6; IR (KBr) v: 3447, 3204, 1669, 1618, 1515, 1459, 1434, 1401, 1298, 1252, 1174, 1034, 728 cm^{-1}; MS (ESI) m/z: 506.2 $[M+H]^+$, 528.2 $[M+Na]^+$, 544.0 $[M+K]^+$; HRMS calcd for $C_{21}H_{18}Cl_2N_5OS_2$ 506.0279, found 506.0279.

3-(3,4-Dichlorophenyl)-1-methyl-*N*-(5-((4-nitrobenzyl)thio)-1,3,4-thiadiazol-2-yl)-1*H*-pyrazole-5-carboxamide (**A26**): Brown powder, yield 53%. m.p. 247～249 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.22 (s, 1H, NH), 8.21 (d, J=8.8 Hz, 2H, ArH), 7.96 (s, 1H, ArH), 7.81 (s, 1H, ArH), 7.74 (s, 2H, ArH), 7.73 (s, 1H, ArH), 7.70 (s, 1H, py-CH), 4.68 (s, 2H, SCH_2), 4.17 (s, 3H, py-CH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ: 163.1, 161.6, 149.9, 142.0, 136.4, 135.3, 131.0, 130.7, 128.8, 124.3, 124.1, 123.9, 121.0, 117.1, 116.2, 107.2, 39.2, 34.7; IR (KBr) v: 3445, 3210, 1669, 1626, 1544, 1519, 1459, 1401, 1350, 1291, 1250, 1097, 1036, 885, 726 cm^{-1}; MS (ESI) m/z: 521.2 $[M+H]^+$, 543.0 $[M+Na]^+$; HRMS calcd for $C_{20}H_{15}Cl_2N_6O_3S_2$ 521.0024, found 521.0026.

4.4 *In vitro* anti-proliferative assay

The *in vitro* cytotoxic activity of compounds **A1**～**A26** against human liver cancer (HepG2) and human gastric cancer (MGC803) was evaluated by the MTT method and the potency was expressed as inhibition rate. A MTT assay was performed to investigate *in vitro* cytotoxic activity. HepG2 and MGC-803 seeded into the 96-well plate (100 μL each well) were incubated at 37 ℃ under 5% CO_2 and 95% O_2 until cell adherence was observed. Cells were exposed to suspension of compounds **A1**～**A26** at different concentrations. After 48 h, 20 mL of MTT with a concentration of 5 mg/mL was added into the 96-well plate and incubated for 4 h at 37 ℃. Then, the supernatant was aspirated, 150 μL of DMSO was added to each well, and the absorbance was measured at 570 nm. The formula for calculating inhibition of cell proliferation was as follows:

Inhibition rate(%)=[OD(control group)−OD(drug group)]/OD(control)×100%

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